Development of a Myeloproliferative Disorder in Beagles Continuously Exposed to $^{90}$Sr

By D. L. Dungworth, M. Goldman, J. W. Switzer and D. H. McKelvie

The term “MYELOPROLIFERATIVE DISORDER” was introduced by Dameshek\1,\2 to embrace a complex array of diseases characterized by irreversible proliferation of one to several of the bone marrow cell lines, and includes such conditions as granulocytic leukemia, myelofibrosis with myeloid metaplasia, polycythemia vera and erythremic myelosis. The interrelationships among the various manifestations have been open to investigation only in human patients, and are still the subject of much debate. Spontaneous myeloproliferative disorders in domesticated animals are rare\3,\4 with the probable exception of the cat, and usually present as granulocytic leukemia. Until recently, therefore, when it was shown that beagles and miniature swine exposed continuously to $^{90}$Sr developed a myeloproliferative disorder,\5 no animal analogy has been available for precise study. Whereas in beagles we have thus far observed only a myeloproliferative disorder,\6 in swine both myeloproliferative and lymphoproliferative disorders have been reported.\7

It has been known for some time that radiation is leukemogenic and that, in particular, bone-seeking radionuclides are effective in inducing leukemias in man and animals.\8 Incidence rates of granulocytic and lymphocytic leukemias were increased in survivors of the Hiroshima and Nagasaki atomic bombing of World War II,\9 and myelofibrosis with myeloid metaplasia has been observed occasionally.\10 Leukemias have also been noted with increased frequency in populations receiving accidental or therapeutic medical X-irradiation,\11 and myeloproliferative disorders have occurred in radium dial luminizers following ingestion of radium-paint mixtures.\12

When beagles are subjected to dietary exposure by $^{90}$Sr from mid-gestation to 1.5 years of age, a significant number of dogs on the higher dose levels have developed a myeloproliferative disorder which in its most active form presents as a granulocytic leukemia. More chronic cases resemble myelofibrosis with myeloid metaplasia (syn. agnogenic myeloid metaplasia). The purpose of this paper is to summarize our observations on beagles to date and to speculate...
Table 1.—Variation with Age of the Number of Beagles at Risk in the Various $^{90}$Sr Dosage Levels

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>$\mu$Ci $^{90}$Sr/day</th>
<th>0.10 to 0.99 Yr</th>
<th>1.00 to 1.49 Yr</th>
<th>1.50 to 1.99 Yr</th>
<th>2.00 to 2.99 Yr</th>
<th>3.00 to 3.99 Yr</th>
<th>4.00 to 4.99 Yr</th>
<th>5.00 to 5.99 Yr</th>
<th>6.00 to 6.99 Yr</th>
<th>7.00 to 7.99 Yr</th>
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</thead>
<tbody>
<tr>
<td>D00</td>
<td>0.00</td>
<td>96</td>
<td>79</td>
<td>71</td>
<td>50</td>
<td>40</td>
<td>6</td>
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<td>D05</td>
<td>0.03</td>
<td>104</td>
<td>88</td>
<td>68</td>
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<td>4</td>
</tr>
<tr>
<td>D10</td>
<td>0.08</td>
<td>56</td>
<td>40</td>
<td>35</td>
<td>28</td>
<td>22</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>D20</td>
<td>0.5</td>
<td>79</td>
<td>67</td>
<td>54</td>
<td>46</td>
<td>41</td>
<td>24</td>
<td>16</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>D30</td>
<td>1.5</td>
<td>83</td>
<td>65</td>
<td>59</td>
<td>59</td>
<td>46</td>
<td>33</td>
<td>13</td>
<td>6</td>
<td>—</td>
</tr>
<tr>
<td>D40</td>
<td>4</td>
<td>67</td>
<td>54(1)*</td>
<td>50</td>
<td>47(2)</td>
<td>37</td>
<td>23</td>
<td>12(1)</td>
<td>10</td>
<td>—</td>
</tr>
<tr>
<td>D50</td>
<td>12</td>
<td>69</td>
<td>54(2)</td>
<td>52(2)</td>
<td>44(3)</td>
<td>31(3)</td>
<td>12</td>
<td>4</td>
<td>1</td>
<td>—</td>
</tr>
</tbody>
</table>

* Number of cases with myeloproliferative disorder is in parentheses.

on the developmental spectrum of this particular form of radiation-induced myeloproliferative disorder.

**Materials and Methods**

$^{90}$Sr Administration

The experimental design utilized requires that the skeleton of beagles be uniformly labeled with $^{90}$Sr. This is achieved by maintaining the appropriate dietary level of $^{90}$Sr, which ranges from $\sim$0.03 to $\sim$12 $\mu$Ci $^{90}$Sr/day (Table 1), from mid-gestation through nursing to 1.5 years of age, at which time the dogs are mature and adult skeletons have been achieved. Logistical considerations necessitated a continuing dosing spectrum with respect...
to time. Thus, the animals at risk at any one time represent the survivors of an initial group of 60 dogs plus the main experimental colony which was developed in two production waves two years apart. Table 1 indicates the decreasing number of animals available at increasing age because of the summation of the above experimental protocol, less animals that were killed or died, and is not to be confused with a colony life table.

**Dosimetry**

Although $^{90}\text{Sr}$ levels remain constant in the diet, the radiation dose rates absorbed by the skeleton are not constant. During the fetal and nursing periods, maternal discrimination against strontium, together with the low level of mineralization and small size of the skeleton, result in initial low dose rates. Following weaning, the dog's skeleton rapidly mineralizes and the absorbed radiation dose increases in a manner roughly proportional to skeletal mineralization.$^{13}$ At the end of the dosing period, the maximum body burden of $^{90}\text{Sr}$ will then be reduced progressively by biologic turnover which is a combination of skeletal remodeling, diffusion, and radioactive decay. Local dose rates consequently decrease in a nonuniform manner proportional to the specific regional mineral turnover rates.$^{14}$ The integrated contribution of these physical, chemical, and biologic factors was utilized in computing the dose rate patterns shown in Figure 1. The beta ray dose from the decay of $^{90}\text{Sr}$ and its short-lived daughter, $^{90}\text{Y}$, is almost entirely absorbed within the skeleton due to its short range in tissue and, therefore, produces minimal irradiation of extramedullary hematopoietic sites.

**Clinical Examinations**

Dogs were given a routine physical examination in alternate weeks; a more complete examination, including a total skeletal radiographic survey, was made annually on the dog's birth date. A complete hemogram was performed every 60 days. Serum samples were obtained every 120 days for chemical analyses. Abnormalities found on any of these occasions led to more detailed and frequent examinations.

Leukocyte and erythrocyte counts were determined by a Coulter Model B electronic particle counter. Hemoglobin determination was performed using Drabkin's solution and a Coleman Jr. spectrophotometer at 540 $\text{nm}$. Hematocrits were determined by microtechnic using the International Model MB8 centrifuge. Air-dried blood smears were stained with Wright's-Leishman stain. One hundred leukocytes were enumerated for the differential count.

Two beagles died during the course of the experiments; 12 others were killed by electrocution when their clinical condition appeared terminal. Since the desire was to observe the unmodified course of the disease, no therapy was given to any of the cases. Terminal bone marrow samples were taken in the later cases after it had been established that a myeloproliferative disorder existed in the colony.

Mean and 2 standard deviations (SD) for total leukocyte counts and hematocrits for control beagles of this colony and for irradiated animals at the highest dose level which did not exhibit the myeloproliferative syndrome are given in Figure 2. Anemia and leukopenia were considered to be present when the respective values for hematocrit and leukocyte count fell below the normal 2 SD limit.

**Necropsy Examinations**

Complete necropsies were performed, including recording of organ weights. Duplicate tissue samples were placed in Bouin's fixative and in 10 per cent neutral formalin. In later cases, impression smears were made from bone marrow, liver, spleen, and lymph nodes and stained with Wright's-Leishman or Giemsa stain. Paraffin-embedded histologic sections were routinely stained with hematoxylin and eosin. Selected sections were stained by Gordon and Sweet's reticulin stain, Giemsa, phloxine-methylene blue, and modified May-Grunwald Giemsa stain.$^{16}$
MYELOPROLIFERATIVE DISORDER

Fig. 2—Mean and 2 SD values of leukocyte and hematocrit for controls and the highest dose level (D50), omitting those with the myeloproliferative disorders.
Fig. 3—Individual lifetime leukocyte and hematocrit values of beagles which developed a myeloproliferative disorder.
MYELOPROLIFERATIVE DISORDER

Fig. 3 (cont'd)
RESULTS

Clinical Findings

Fourteen cases of myeloproliferative disorder have occurred to date, 4 in the 4 \( \mu \text{Ci} \text{SrO} \text{d} \text{ay} (D40) \) dose level and 10 in the 12 \( \mu \text{Ci} \text{SrO} \text{d} \text{ay} (D50) \) level. Distribution of cases with respect to age of beagles and numbers at risk is given in Table 1. Cases first appeared in the one year age group and the approximate annual incidence increased thereafter.

Onset of the disorder was indicated by a drop in the hematocrit which then followed a progressive or precipitous course (Fig. 3). Cases fell into two major groups, those with an acute episode of less than 100 days and those with a more chronic course of 240 to 624 days (Table 2). In only one instance (D40F81) was there a temporary remission in the anemic state.

Erythrocyte morphology was abnormal in most of the dogs (Table 2). Prominent poikilocytosis, anisocytosis and hypochromasia were seen in the majority, and 4 had terminal macrocytosis. In few dogs was there any evidence of polychromasia, nucleated red cells or other signs of erythroid regeneration.

An inconsistent pattern was observed in the concentration of peripheral blood leukocytes. Although all of the dogs initially exhibited the characteristic dose-related, radiation-induced leukopenia, most of the cases subsequently developed wide fluctuations in the leukocyte count (Fig 3). Terminaly, a moderate but significant leukocytosis was observed in five of the fourteen dogs affected. The highest terminal leukocyte count (D50M32) was 38,900/mm\(^3\) (Table 2). The remaining dogs had low normal or leukopenic counts, but only 2 were below the lower 2 SD limit for the main group of D50 dogs. All dogs had a slight to severe left shift of the granulocytic series in the peripheral blood. Two dogs (D50M32 and D40F81) had myeloblasts in peripheral blood during the terminal stages (Fig. 4). The degree of immaturity of the granulocytes was not related to the leukocyte count. The blood of one dog (D50F80) contained bizarre giant neutrophils.

Although platelet counts were not performed, blood smears from all of the beagles had an extreme decrease in number of platelets. The finding at post-mortem examination of petechiae, ecchymoses and gastrointestinal bleed-
Table 2.—Terminal Hemograms of ⁹⁰Sr-Irradiated Beagles with a Myeloproliferative Disorder

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Age at Birth (Yrs.)</th>
<th>Duration of Anemia (Days)</th>
<th>Hct (%)</th>
<th>Total Leukocytes (×10⁹)</th>
<th>Myeloblasts</th>
<th>Progranulocytes</th>
<th>Leukocyte Differential (in %)</th>
<th>Lymphocytes</th>
<th>Metamyelocytes</th>
<th>Band Neutrophils</th>
<th>Segmented Neutrophils</th>
<th>Monocytes</th>
<th>Eosinophils</th>
<th>Unclassified</th>
<th>Anisocytosis- (A)</th>
<th>Poikilocytosis (P)</th>
<th>Polychromasia (M)</th>
<th>Hypochromasia (H)</th>
<th>Estimated Platelets (×10⁹)</th>
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<tbody>
<tr>
<td>D50M32</td>
<td>2.3</td>
<td>24</td>
<td>17</td>
<td>38,900</td>
<td>13</td>
<td>26</td>
<td>8</td>
<td>10</td>
<td>18</td>
<td>22</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D40M76</td>
<td>2.3</td>
<td>&lt;4</td>
<td>1</td>
<td>5,200</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>69</td>
<td>21</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D50F29</td>
<td>3.2</td>
<td>83</td>
<td>19</td>
<td>29,400</td>
<td>1</td>
<td>14</td>
<td>14</td>
<td>6</td>
<td>7</td>
<td>49</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D50M56</td>
<td>1.2</td>
<td>71</td>
<td>23</td>
<td>25,000</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>18</td>
<td>49</td>
<td>10</td>
<td>0</td>
<td>6</td>
<td>14</td>
<td>0</td>
<td>A, P +</td>
<td>H +</td>
<td>&lt;100,000</td>
<td></td>
</tr>
<tr>
<td>D50M33</td>
<td>1.8</td>
<td>41</td>
<td>8</td>
<td>6,900</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>81</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>A, P ±</td>
<td>-</td>
<td>+</td>
<td>&lt;100,000</td>
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<tr>
<td>D50M39</td>
<td>2.7</td>
<td>61</td>
<td>9</td>
<td>16,200</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>6</td>
<td>6</td>
<td>42</td>
<td>22</td>
<td>18</td>
<td>2</td>
<td>0</td>
<td>A, P ±</td>
<td>-</td>
<td>+</td>
<td>&lt;100,000</td>
<td></td>
</tr>
<tr>
<td>D50F14</td>
<td>1.5</td>
<td>59</td>
<td>5</td>
<td>3,300</td>
<td>0</td>
<td>0</td>
<td>18</td>
<td>4</td>
<td>2</td>
<td>14</td>
<td>62</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>A, P ++</td>
<td>H +</td>
<td>+</td>
<td>&lt;500</td>
<td></td>
</tr>
<tr>
<td>D50F90</td>
<td>1.3</td>
<td>108</td>
<td>14</td>
<td>3,500</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>43</td>
<td>37</td>
<td>1</td>
<td>0</td>
<td>12</td>
<td>A, P ++</td>
<td>F +</td>
<td>H ++</td>
<td>&lt;500</td>
<td></td>
</tr>
<tr>
<td>D50F40</td>
<td>1.4</td>
<td>77</td>
<td>16</td>
<td>4,600</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>65</td>
<td>30</td>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>A, P ++</td>
<td>H +</td>
<td>+</td>
<td>&lt;100,000</td>
<td></td>
</tr>
<tr>
<td>D50M64</td>
<td>3.5</td>
<td>624</td>
<td>9</td>
<td>7,100</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>21</td>
<td>22</td>
<td>45</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>A, P ±</td>
<td>-</td>
<td>+</td>
<td>&lt;500</td>
<td></td>
</tr>
<tr>
<td>D50F30</td>
<td>3.6</td>
<td>412</td>
<td>10</td>
<td>22,300</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>81</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>A, P +</td>
<td>M, H +</td>
<td>&lt;100,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D50M41</td>
<td>3.4</td>
<td>240</td>
<td>11</td>
<td>5,700</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>28</td>
<td>38</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>A, P ±</td>
<td>-</td>
<td>+</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>D40M05</td>
<td>5.9</td>
<td>568</td>
<td>7</td>
<td>6,100</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>12</td>
<td>6</td>
<td>30</td>
<td>47</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>A, P +</td>
<td>M + +, H +</td>
<td>&lt;100,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D40F81</td>
<td>2.1</td>
<td>390</td>
<td>3</td>
<td>4,100</td>
<td>2</td>
<td>3</td>
<td>17</td>
<td>8</td>
<td>25</td>
<td>28</td>
<td>11</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>A, P ++</td>
<td>M, H +</td>
<td>&lt;500</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Indicates dosage level, sex, and individual dog number. Cases are ranked according to severity (see Text and Table 3).

† This dog died of a severe hemorrhagic anemia 4 days after a hemogram showing a Hct of 45 and cell values as listed.

‡ Abnormal myelocytes.

§ Including 6% plasma cells.

|| ± marginal; + slight; ++ moderate; +++ severe.
Fig. 4.—Peripheral blood smear from dog D50M32 showing a cluster of myeloblasts with a promyelocyte and 2 neutrophils (Wright's-Leishman, × 1700).

ing in some of the dogs indicated the presence of a hemorrhagic disorder related to the thrombocytopenia.

The general condition of the beagles deteriorated as the anemia progressed, the main clinical signs being depression, weakness, anorexia and progressive weight loss.

**Macroscopic Lesions**

All carcasses were anemic, and they were generally either in poor condition or frankly emaciated. The two dogs that died did so because of acute hemorrhage. In one (D40M76), there were severe intestinal hemorrhage, subcutaneous hemorrhage from a recent jugular venipuncture, and widespread pericardial, epicardial and endocardial ecchymoses. The other (D50M64) had hemoperitoneum resulting from several ruptures in the hepatic and splenic capsules. Four of the dogs that were killed had scattered subcutaneous ecchymotic and petechial hemorrhages, and a fifth had bled excessively from a traumatic tongue lesion.

Splenomegaly was the most notable feature of the disease process in all but the four least affected dogs, and correlated well with subsequent histologic assessment of the total extent of lesions in each dog (Table 3). Grossly affected spleens weighed 2 to 10 times more than normal, and on section were homogenous, brick red to dark red, fleshy and slightly moist. Slight to moderate lymph node enlargement was also usually observed, the degree of enlargement paralleling that of the spleen, but at a much lower level. Nodes of most dogs were tan to rust-red, particularly in their medullas. Only in
MYELOPROLIFERATIVE DISORDER

Fig. 5.—Femoral metaphyseal bone marrow from dog D40M76 indicating dense hypercellularity (hematoxylin and eosin, × 52).

dog D50M32 was there appreciable hepatomegaly, the liver being approximately twice normal weight (690 Gm.) and having a fine white stippling. This same dog had widespread, small, irregular foci of grey consolidation in its lungs.

Gray-pink to reddened bone marrow had replaced fatty marrow in shafts of long bones. In addition, there was increased thickness and density of cortical bone in the diaphyses of long bones, and to a lesser degree in other portions of the skeleton. This was regularly observed in dogs exposed to high level 90Sr feeding, whether or not they developed the myeloproliferative disorder.

No other gross lesions of significance were detected.

Microscopic Lesions

Features common to all 14 beagles were extensive granulocytic proliferation in the bone marrow, with concomitant depletion of erythroid and megakaryocytic elements, and extramedullary granulopoiesis which was predominantly in the spleen and to a lesser extent in liver, lymph nodes, kidney and a variety of other organs. Taken as a group, the beagles presented a continuous spectrum of severity of these features, as indicated in Table 3, in which cases are ranked according to the combined assessment of extent of granulopoiesis in bone marrow and spleen, and the degree of its maturation arrest. Various degrees of the latter were present in bone marrow of all but the lowest ranked dog, and were detected in 9 of the 14 spleens. Megakaryocytes were generally rare or few in number in the sites involved, and the only instances in which the proportions of hemopoietic cell lines resembled those of normal bone marrow were in spleens of the three lowest ranked dogs. The order of listing for hemogram data
Table 3.—Extent of Granulopoiesis in 50\textsuperscript{Sr}.

<table>
<thead>
<tr>
<th>Dog Number</th>
<th>Cellularity</th>
<th>Fibrosis</th>
<th>Granulocytic Maturation Arrest</th>
<th>Quantity of Erythropoiesis and Megakaryocytes</th>
<th>Spleen *</th>
<th>Weight (Gm.)</th>
<th>Granulocytic Maturation Arrest</th>
<th>Quantity of Erythropoiesis and Megakaryocytes</th>
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<tbody>
<tr>
<td>D50M32</td>
<td>++++</td>
<td>N</td>
<td>+</td>
<td>+++</td>
<td>300</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D40M76</td>
<td>++++</td>
<td>N</td>
<td>+</td>
<td>+++</td>
<td>69</td>
<td>+++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50F29</td>
<td>++++</td>
<td>N</td>
<td>+</td>
<td>+++</td>
<td>310</td>
<td>+++</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>D40M56</td>
<td>++++</td>
<td>N</td>
<td>±</td>
<td>+++</td>
<td>178</td>
<td>+++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50M33</td>
<td>+++</td>
<td>±</td>
<td>++</td>
<td>+++ (slight fibrosis)</td>
<td>113</td>
<td>++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50M39</td>
<td>+++</td>
<td>±</td>
<td>++</td>
<td>+++</td>
<td>86</td>
<td>++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50F14</td>
<td>+++</td>
<td>±</td>
<td>++</td>
<td>+++ (slight fibrosis)</td>
<td>95</td>
<td>++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50F80</td>
<td>++++</td>
<td>N</td>
<td>+</td>
<td>+++ (slight fibrosis)</td>
<td>113</td>
<td>++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50F40</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>+++ (slight fibrosis)</td>
<td>92</td>
<td>++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>D50M64</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>55</td>
<td>N</td>
<td>+</td>
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</tr>
<tr>
<td>D50F30</td>
<td>++++</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>35</td>
<td>N</td>
<td>± (slight)</td>
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</tr>
<tr>
<td>D50M41</td>
<td>+++</td>
<td>N</td>
<td>+</td>
<td>+</td>
<td>Normal (&lt;35)</td>
<td>N</td>
<td>+</td>
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<tr>
<td>D40M05</td>
<td>+++</td>
<td>±</td>
<td>+</td>
<td>+</td>
<td>Normal N</td>
<td>+</td>
<td>+</td>
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<tr>
<td>D40F81</td>
<td>+++</td>
<td>N</td>
<td>−</td>
<td>+</td>
<td>Normal N</td>
<td>+</td>
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</tbody>
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* Quantitative assessment denoted by: − less than normal; N normal or none; ± marginal increase; + slight increase; ++ moderate increase; +++ severe increase; ++++ maximal increase.

(Table 2) is that developed in Table 3. The correlation between morphologic severity and clinical course of the disease (duration of anemia) is excellent. Amplification of histologic findings within the various organs follows.

**Bone marrow.** All marrows had granulocytic proliferation with depletion of erythroid precursors and megakaryocytes. Table 3 indicates that the extent of this ranged from virtual absence of erythropoietic elements and megakaryocytes in the most severely affected marrows, down to the least affected with an M:E ratio, as determined from an impression smear, of 30:1, and almost normal numbers of megakaryocytes. Marrows with maximal (4+) cellularity were densely crowded to the point of complete obliteration of fat in samples from the proximal femoral diaphysis (Fig. 5), and those with 3+ cellularity were only slightly less crowded.
There was maturation arrest of the granulocytic series in all but the lowest ranked dog (Table 3). Immature cells (mostly myeloblasts) predominated in those with moderate to severe arrest and mitotic indices were high, ranging from approximately 5/high-power field (HPF) for dog D50M46 to 10/HPF for D40M76 (Fig. 6). Occasionally there were blast cells with folded, irregularly shaped or double nuclei. The correlation between the degree of maturation arrest and the extent of granulocytic proliferation is good, with the exception of dogs D50F29 and D40M56.

Myelofibrosis was present in 6 marrows. In 4 it was marginal, in one slight and in one moderate, where the interlacing bundles of collagenous connective tissue occupied approximately 50 per cent of the sectional area (Fig. 7).
Spleen. Histologic pattern correlated well with splenic weight, as would be expected. Extensive involvement (4+ and 3+) produced complete or almost complete obliteration of architecture. There was a moderate to severe maturation arrest, the cell population consisting largely of blast forms with high mitotic index (Fig. 8). Many vessels were partially or completely filled...
MYELOPROLIFERATIVE DISORDER

Fig. 8.—Spleen of dog D50M32 showing predominance of blast cells with high mitotic index (hematoxylin and eosin, × 800).

with similar cells, and there were regions of capsular and trabecular invasion. Erythropoietic cells were rare or inconspicuous, and megakaryocytes were also scarce.

A clear line of demarcation was present between the degree of splenic

Fig. 9.—Trabecular infiltration of a predominantly myeloid cell population in the medulla of a lymph node from dog D50F14 (hematoxylin and eosin, × 240).
ranked dogs (Table 3). The quantity of granulopoiesis was not great, normal splenic architecture was retained, and the proportions of erythropoietic, granulopoietic and megakaryocytic cells were similar to those seen in normal bone marrow. There was no detectable maturation arrest in the granulocytic cell series. A few myeloblasts could still be seen in splenic vessels, however.

**Lymph nodes.** Nodes were considerably less affected than corresponding spleens, and the nodes of any one dog varied in their degree of involvement. Most advanced lesions (3+) were present in nodes of D50M32 where in some instances there were practically complete obliteration of architecture and extensive pericapsular invasion. The predominant blast cell population resembled those seen in the spleen and bone marrow of this dog. Less prominent lesions occurred in the majority of dogs, and consisted of differing degrees of widening and obliteration of medullary cords by mixed blast to polymorphonuclear cell populations. There was associated infiltration into trabeculae (Fig. 9) with spill over into the sinuses, and usually regions where capsules and pericapsular tissues were infiltrated. Even in the marginally affected nodes, where sometimes it was difficult to detect granulopoietic elements against a background of lymphoid and plasma cell proliferation, there were regions of mild trabecular and capsular infiltration by a predominantly polymorphonuclear cell population. As in the spleen and bone marrow, the less affected the nodes, the more prominent was a minor component of erythroid and megakaryocytic cells.

**Liver.** The extent of perivenous and sinusoidal myeloid infiltration (3+) in the liver of D50M32 was sufficient to produce moderately severe architectural distortion and parenchymal atrophy. The central and sublobular veins and associated centrilobular regions were mainly affected, and portal regions were relatively spared. The proportion of blast and polymorphonuclear forms

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Fig. 10.—Severe neoplastic invasion in the lung of D50M32, mainly in perivascular regions (hematoxylin and eosin, × 45).
involvement as just described and the mildly affected spleens of the 5 lowest varied, the former predominating in sinusoids and the latter predominating within the walls of affected veins. Subendothelial accumulation in some veins produced narrowing of their lumina. A few erythroid cells were present. Lesions in D50F29 were similar but less pronounced.

Livers with slight involvement presented a fairly uniform picture of dense aggregates of myeloid cells forming collars within and around the majority of central and sublobular veins. These reached 100 to 200μ in width and endothelial elevation often caused luminal narrowing. Blast cells usually predominated, except within dense connective tissue sites. There was also a generalized slight increase of cells in sinusoids, either singly or in small clusters. Small numbers of megakaryocytes and erythroid cells were seen. Portal regions were occasionally minimally affected.

Marginal involvement in livers of dogs at the low end of the spectrum was manifested by a few small clusters of cells, mostly polymorphonuclear cells, within centrilobular or portal regions.

Periacinar lipidosis, sometimes with superimposed terminal necrosis, was a frequent feature in livers of dogs with severe anemia.

Lung. There was severe myeloid infiltration in the lung of D50M32 with Infiltrations were heaviest around vessels, with consequent pronounced involvement of peribronchial tissues also, and in subpleural regions. Alveolar septal involvement, other than by extension from adjacent perivascular sites, was more moderate. The predominant blast cell population resembled those seen elsewhere in this dog.

Fig. 11.—Slight accumulation of mixed blast and polymorphonuclear cells in the choroid coat of the eye of dog D50M41 (ranked twelfth) (hematoxylin and eosin, × 800).
The degree of myeloid infiltration in D50F29 and D50M39 was insufficient to produce consolidation. There was a generalized, moderate accumulation of approximately equal proportions of blast cells and mature granulocytes within alveolar capillaries which produced an obvious increase in septal cellularity. Similar cells were present in larger vessels. Only occasional cell clusters were present in extravascular sites such as peribronchial and subpleural interstitium. A slightly lesser degree of this same pattern constituted the + degree of involvement, whereas careful examination was necessary to reveal the occasional intravascular blast cells of marginal involvement.

**Kidney.** This was the least affected of the organs mentioned thus far. At best the infiltration was slight, and consisted of small interstitial foci of mixed granulocytic maturation forms and sometimes erythroid precursors also. Large blast cells usually predominated and occasionally were present as an almost pure population. The predilection site for infiltrations was within and adjacent to the wall of superficial cortical veins. Other common sites were periglomerular, around arcuate vessels, within the renal capsule, and around small vessels in the hilar connective tissues. The same pattern of location of occasional sprinklings of cells was seen in the minimally affected kidneys.

**Other organs.** Mild to minimal granulocytic infiltrations occurred in a wide variety of organs, the number and nature of which are indicated in Table 3. The infiltrations generally consisted of light to modest accumulations of cells, which were usually at least 50 per cent blast forms, in connective tissues surrounding small arteries and veins within the interstitium of affected organs. The degree of involvement of these organs, since it was small, varied little between cases at the high and low end of the spectrum. Special mention must be made of the slight involvement of the choroid (Fig. 11) in 5 of 8 eyes available for examination, and of portions of choroid plexus in 5 of 6 for which specimens were available. There were also erythroid cells and sometimes megakaryocytes in the latter site.

**DISCUSSION**

Continuous exposure of beagles to $^{90}$Sr from mid-gestation to 1.5 years of age induced a myeloproliferative disorder with an apparently dose-related increase in incidence. The difference in incidence between the two levels (4 in the 4 μCi/da group and 10 in the 12 μCi/da group) paralleled the threefold difference between them in $^{90}$Sr dose rate. Whether this relationship will hold for other dosage rates remains to be seen.

The 14 cases in the series present a continuous morphologic spectrum. The most acute cases behaved as granulocytic leukemias while the chronic ones were not frank leukemias but bore some resemblance to myelofibrosis with myeloid metaplasia (MMM) in man. Clear-cut separation within the spectrum is not possible on the basis of data available currently. Although the exactness of the analogy to the difficulty in separation between granulocytic leukemia and MMM in man$^{16-18}$ is debatable and subject to further study, the parallels are sufficiently close to deserve additional comment.

Typically, MMM in man has a chronic course and the triad of leukoerythroblastic anemia, splenomegaly and myelofibrosis, although in early cases
the bone marrow might be hypercellular and the spleen not enlarged. It is generally agreed that although there might be a predominance of one cell line in bone marrow, spleen or other sites of involvement, a severe maturation arrest is not characteristically found. According to criteria developed in man, the 5 beagles at the lower end of the spectrum had a condition resembling MMM in the following features: chronic clinical course (240 to 624 days); low or only slightly elevated leukocyte count with shift to the left; anemia with prominent anisocytosis and poikilocytosis; terminal thrombocytopenia; hyperplastic marrow with little or no shift to the left; and a mild myeloid metaplasia in the spleen and elsewhere with proportions of cell lines resembling normal bone marrow. The absence of paucity of myelofibrosis could perhaps be explained by the relatively short duration combined with low intensity of marrow “insult,” but the degree of exclusion of erythropoietic and megakaryocytic elements in the bone marrow would be unusual for MMM in man. The absence of a pancytopenia in these beagles would therefore seem at present to distinguish their disorder from MMM in man.

At the other end of the spectrum, several dogs had lesions that were more typical of granulocytic leukemia in that they had an acute clinical course (<100 days) and massive granulocytic proliferation in bone marrow and spleen with overwhelming preponderance of primitive cells at one or both of these sites. They did not have myelofibrosis. The most severely affected dog also had extensive infiltrations causing architectural distortions or obliteration in liver, lymph nodes and lung. Of the conventional morphologic criteria for differentiating MMM and granulocytic leukemia, the preponderance of a primitive cell of the granulocytic series (“leukemic hiatus”) in the latter is generally accepted as being the most important. The blood picture in these dogs would not clearly differentiate between MMM and granulocytic leukemia, except probably for the degree of left shift in the dog D50M32. None of them had a particularly high leukocyte count, possibly because of the acute course. In the few published reports, dogs with granulocytic leukemia have usually had leukocyte counts exceeding 100,000; counts of approximately 16,000 and 30,000 have been recorded. The degree of leukocytosis in the beagles might have been higher if supportive treatment for the anemia had allowed them to live longer since the very high counts occur terminally.

Cases occupying the middle portion of the spectrum possessed features of both MMM and granulocytic leukemia to a degree that we would not wish to attempt to pigeon-hole them. This is in accord with the fact that irrespective of whether the opinion is held that the two conditions are distinct entities or merely divergent manifestations of the same disorder, all are agreed that there are cases in between that defy classification. Where one attempts to draw lines of demarcation in our spectrum no doubt depends to a large extent on one’s conceptual approach.

The presence of the Philadelphia (Ph1) chromosome, absence or decrease in leukocyte alkaline phosphatase, and elevation of serum vitamin B12 level and of unsaturated vitamin B12 binding capacity have all been used to separate chronic granulocytic leukemia from MMM in man. These facets have not yet been investigated in beagles, although they will be in cohorts now enter-
ing the age at which the disorder is expected to occur. They will not necessarily be of as much value as in man, however, since even if a marker chromosome exists it will be difficult to document in view of the fact that the dog possesses 76 small acrocentric autosomes, and reports to date indicate that normal dog neutrophils contain little or no alkaline phosphatase.\(^{35-37}\)

Considerations of pathogenesis will remain purely speculative until serial studies, especially kinetic ones, can be performed during development of the disorder. Continual marrow irradiation causes a chronic leukopenia in all of the beagles on high dose levels, and it is against this background that some dogs in the two highest dose levels, and therefore those with the most pronounced leukopenia, develop the myeloproliferative disorder. The absence of anemia in the high dosage level dogs, in contrast to leukopenia, is probably accounted for by rapid development of extramedullary erythropoiesis (sites largely removed from radiation effects from the bone-seeking radionuclide), such as occurs in \(^{89}\)Sr-treated mice.\(^{38}\) Some support for this contention is provided by the finding of slightly increased amounts of hematopoiesis in the spleens of at least 4 of 8 dogs that died for reasons unrelated to a myeloproliferative disorder, in the absence of obvious bone marrow changes.

Our hypothesis, which is in line with the reasoning of Dameshek,\(^{39}\) Bierman,\(^{40}\) Cronkite,\(^{41}\) and others,\(^{42}\) is that continual, low-level bone marrow irradiation, besides its general destructive effect causes the emergence of clones of granulocytic cells possessing differing and probably changing degrees of abnormality. Certainly in the cases we observed there were various degrees of maturation defect, with failure of normal cell regulation both within and between the dividing and nondividing granulopoietic cell pools. These observations, as well as the delay between initial \(^{89}\)Sr exposure and the onset of the disorder, can best be explained in the basis of multiple causal events being required, at least for the most acute and primitive manifestations. To what extent mutation, feedback deletion, viruses and immunologic factors, to name just some of the more plausible ones, might be involved is not yet known. The effectiveness of the dose rates utilized might induce a greater cell “injury” to death ratio, which might in turn favor neoplastic transformation. Since the hematopoietic precursor cells are irradiated throughout the growth; differentiation and development ages it is possible that an early age-sensitive synergism might be an additional factor in the leukemogenic process.

The low, normal, or only slightly raised leukocyte numbers in the peripheral circulation in spite of extensive granulocytic proliferation in all dogs, indicates ineffective maturation and release mechanisms. The chronic neutropenia itself might place a further proliferative stimulus on granulopoiesis through lack of normal feedback control.\(^{40}\) The absence of high leukemic counts in spite of an extensively proliferating primitive cell population in some dogs is in accord with the observation that cells which have lost the ability to divide are released preferentially into the blood.\(^{43}\)

Part of the direct effect of \(^{89}\)Sr on bone is to produce pachyostosis, with partial obliteration of marrow spaces. Although the role of this has to be considered in the production of myeloid metaplasia, it is unlikely to be of
undue importance, partly because it primarily effects the diaphyses of long bones and the estimated reduction of total marrow space is <20 per cent.44

The onset of anemia, assuming initial extramedullary compensation, is probably due to a combination of factors. The excellent correlation between acuteness of clinical course and the degree of active primitive granulocytic cell proliferation indicates the importance of overcrowding and possibly diversion of a common progenitor pool. There is also the possibility that red blood cells produced at extramedullary sites have decreased life span. Terminally, a hemorrhagic episode of consequence is superimposed in some dogs, as a result of thrombocytopenia.

The occurrence in the beagles exposed to $^{90}$Sr of a spectrum of myeloproliferative disorders which manifest as granulocytic leukemia in the acute form and resemble MMM in the chronic form is not altogether remarkable. The close relationship between them has been stressed on the basis of observations on disease in man, whether of unknown etiology or following bone marrow damage such as by benzol and irradiation. The importance lies in that this is an experimental situation which provides a readily reproducible model for study of fundamental aspects of radiation-induced granulocytic leukemia and which might concurrently shed light on the relationship between granulocytic leukemia and MMM.

**Summary**

In a continuing study of the toxicity of $^{90}$Sr in beagles exposed continuously from mid-gestation to 1.5 years of age, a myeloproliferative disorder has arisen in dogs fed the two highest dose levels, 12 and 4 $\mu$Ci $^{90}$Sr/day, respectively. Ten cases have occurred thus far at the highest level and 4 at the second highest, the difference in incidence being approximately equal to the threefold difference in dose rate. Age at onset has ranged from 14 to 72 months, and cumulative dose rates up to the time of death have varied from about 1,000 to 10,000 rads.

The 14 cases of myeloproliferative disorder present a morphologic spectrum which manifest in the acute form as granulocytic leukemia and in the chronic form resemble myelofibrosis with myeloid metaplasia (MMM). Anemia, poikilocytosis, anisocytosis, hypochromasia and terminal thrombocytopenia were constant features. Terminal leukocyte counts ranged from 3,300 to 38,900 cells/mm$^3$, with various degrees of shift to the left. Splenomegaly was the salient gross finding in most dogs. Major histologic lesions were pronounced granulocytic proliferation in bone marrow and spleen, with concomitant erythroid and megakaryocytic depletion. Liver, lymph nodes and a variety of other organs had less extensive and less frequent involvement. In the most acute cases, bone marrow and spleen contained cell populations composed almost entirely of blast cells.

This experimental situation should serve to shed light on fundamental aspects of radiation-induced granulocytic leukemia and might help to clarify the relationship between granulocytic leukemia and MMM.
SUMMARIO IN INTERLINGUA

In un studio continue del toxicitate de 90Sr in canes (beagles) exponite continuemente a ille agente ab gestation medie usque ad le etate de 1,5 annos, le disveloppamento de un disordine myeloproliferative esseva constatate in le gruppos de animales tractate al duo plus alte nivellos, i.e., de 12 e 4 μCi 90Sr per die, respectivemente. Usque al momento presente, 10 casos ha occurrite al nivello le plus alte e 4 al nivello secundemente le plus alte. Le differentia es approximativamente equal a tres vices le differentia in dosage. Le etate al tempore del declaration del disordine ha variate inter 14 e 72 menses. Le dosage cumulative usque al tempore del morte del animal ha variate inter approximately 1.000 e 10.000 rad.

Le 14 casos de disordine myeloproliferative presenta un spectro morphologic que se manifesta in le forma acute como leucemia granulocytic e in le forma chronic como un condition simile a myelofibrosis con metaplasia myeloide (MMM). Anemia, poikilocytosis, anisocytosis, hypochromasia, e thrombocytopenia terminal esseva characteristicas constantes. Le terminal numeration leucocytic variava inter 3.300 e 38.900 cellulars per mm³, con varic grados de devaliatione in le sinistra. Splenomegalia esseva le plus prominentes constatation macroscopic in le majoritate del canes. Le major lesions histologic esseva un pronunciata proliferation granulocytic in le medulla ossee e le splen, con concomitante depletion erythroide e megakaryocytic. Le hepate, le nodos lymphatic, e un varietate de altere organos monstrava un minus extense e minus frequente affection. In le majoritate del casos acute, le medulla ossee e le splen contineva populationes cellular componite quasi exclusivamente de blastocytos.

Iste constatationes experimental promitte illuminar certe aspectos fundamental de leucemia granulocytic inducite per radiation. Illus va possibilemente esser de adjuta in clarificar le relation inter leucemia granulocytic e MMM.

ACKNOWLEDGMENTS

We are grateful for the technical assistance of W. Gee, D. Yates and co-workers, Carolyn Crowder, H. Miller, Shirley Coffelt and K. Shiomoto. The contributions of Drs. L. K. Bustad, A. C. Andersen, O. W. Schalm, J. E. West and J. F. Wright are greatly appreciated.

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Development of a Myeloproliferative Disorder in Beagles Continuously Exposed to $^{90}$Sr

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